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Evaluation of the Effectiveness of a Soil Treatment Using Calcium Carbonate Precipitation from Cultivated and Lyophilized Bacteria in Soil's Compaction Water

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Abstract: Microbial-induced carbonate precipitation (MICP) is a bio-inspired solution where bacteria metabolize urea to precipitate. This carbonate acts as a bio-cement that bonds soil particles. The existing framework has focused mainly on applying MICP through infiltration of liquid bacterial solutions in existing soil deposits. However, this technique is inefficient in soils with high fines content and low hydraulic conductivity, and thus few studies have focused on the use of MICP in fine soils. The main objective of this study was to evaluate the effect of MICP applied to compaction water in soils containing expansive clays and sandy silts. This approach searches for a better distribution of bacteria, nutrients, and calcium sources and is easy to apply if associated with a compaction process. In soils with expansive minerals, the effect of MICP in swelling potential was explored at laboratory and field scales. In sandy silts, the evolution of the stiffness and strength were studied at the laboratory scale. The treatment at the laboratory scale reduced the swelling potential; nevertheless, no significant effect of MICP was found in the field test. In sandy silts, the strength and stiffness increased under unsaturated conditions; however, subsequent saturation dissolved the cementation and the improvement vanished.

Keywords: microbial-induced carbonate precipitation; soil improvement; expansive clays

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1. Introduction

Soil improvement has become a geotechnical alternative for facing challenges in construction and infrastructure design. Expansion of clays, low shear strength, and high settlements are common problems in geotechnical engineering [1]. The shear strength of soils can be increased by applying cementing additives in the material to bring reinforcement to the soil [2]. On the other hand, compaction mitigates undesired settlements, reducing the void ratio and increasing stiffness [2].

Soils with expansive clays are materials whose volume changes as a function of its moisture [3,4]. Minerals with large specific surfaces, such as smectites, generate expansion on soils and, eventually, damages on structures [1]. The structural problems generated in constructions founded on soils with expansive clays are mitigated through stabilization techniques. A way to control the expansivity of soils is by reducing the repulsive forces between particles, generating a more stable packing of the soil minerals through the inclusion of agglomerants or cementitious products. For instance, Lu et al. [5] evaluated the effect of biochar and coal fly ashes mixed in prior to compaction in the expansiveness of remolded soils. The mixtures were prepared with different proportions of treatments; both additives bonded the micro-aggregates to form macro-aggregates, which stabilized the fabric of the material and reduced free swelling by around 50%. Jamsawang et al. [6]

studied the effect of soil–cement mixtures in the behavior of soils with expansive minerals prior to compaction. They tested several combinations with different proportions of cement. As a result, the bonds created reduced the size of the pores stabilizing the fabric, obtaining a reduction of free swelling up to 90% in the most favorable case. Similarly, Dayioglu et al. [7] generated composites of soil with expansive clays with several cementitious products, obtaining a reduction in swelling pressure and expansion.

Current improvement techniques are helpful and practical; however, they increase project costs and may not be environmentally friendly. As an alternative, there are bio-inspired treatments that can help to reduce the impact of greenhouse emissions and have lower environmental impact [8]. For instance, bio-mineralization is a process developed by bacteria and plants with low emissions of carbon dioxide (CO_2) to the atmosphere. As part of their metabolism, plants and bacteria precipitate carbon into calcium carbonate ($CaCO_3$) [9], which can act as a cementing agent. In particular, microbial-induced carbonate precipitation (MICP) is a bio-inspired soil cementing technique that increases the stiffness and shear strength of soils [10] and additionally reduces the permeability [11] with lower environmental cost [12]. In MICP, microorganisms induce carbonate precipitation metabolizing urea. During the metabolizing process, known as urea hydrolysis, bacteria decompose one mole of urea and three moles of water into two moles of ammonium, two hydroxides, and carbonate dioxide. The metabolic activity of bacteria changes the pH, modifying the ammonium to ammonia, as well as carbon dioxide and hydroxyl to carbonate anion. The presence of calcium ions leads to an equilibrium reaction where carbonate and calcium generate calcium carbonate [9,10,13]. Figure 1 illustrates the precipitation process.

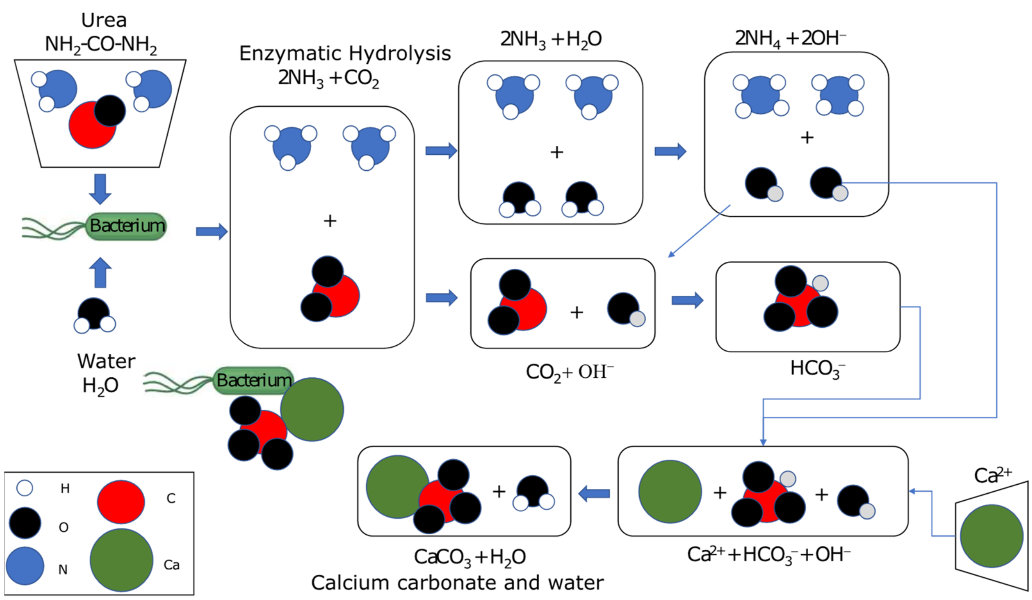


Figure 1. Microbial-induced calcium carbonate precipitation. Urea hydrolysis to carbonic acid takes 18 h to be achieved, while the reaction between carbonic acid and calcium ions occurs instantaneously for practical purposes.

Microbial-induced calcium carbonate can precipitate with various polymorphic forms: calcite, aragonite, vaterite, and amorphous calcium carbonate. Calcite is the most stable and abundant carbonate mineral and has a rhombohedral structure, whereas aragonite is an orthorhombic unit cell [13]. Vaterite is a hexagonal unit cell and is unstable under ambient conditions [14]. Amorphous carbonate is the most common carbonate produced by microorganisms and can evolve to more stable structures under supersaturation conditions and changes in the pH [15]. The main factors affecting the quantity and quality of carbonate precipitation are the cultivation of bacteria [16], bacterial concentration, minerals of soils, pH, the calcium source concentration, and the treatment method [12,17,18].

Several researchers have reported data on sandy soil improvement through MICP. For instance, DeJong [10] showed that the increment of undrained shear strength and the shear wave velocity of Ottawa sand treated with MICP by injection can be larger than treatment with gypsum. Montoya and DeJong [19] injected bacterial solutions through Ottawa sand samples and found that shear wave propagation velocity was duplicated, even with low cementation proportions (over 1%). With increased cementation (concentrations about 3%), the soil reached a stiffness similar to rocks. Additionally, Cheng et al. [20] showed the influence of water content in the efficiency of MICP treatment, concluding that partial saturation promotes the generation of bonds between particles due to the precipitation of calcite carbonate in the meniscus. Jiang et al. [21] showed that MICP reduces the internal erosion of sand–clay mixtures, and the effect was dominated mainly by the precipitated $CaCO_3$.

In clayey soils, Cardoso et al. [22] studied the chemical interaction between bacterial activity and clay minerals. They compared the effect of MICP treatment in sands with mixtures of sand and kaolin. Samples were submerged in bacterial solution for 21 days, and they found that a more significant proportion of bio-cementation in clays is required. However, the particles agglomerated and an increase in tensile strength was observed. On the other hand, Li et al. [23] described the behavior of soil with expansive clays treated with fly ashes and MICP by mixing the product prior to compaction. They found that while fly ashes stabilize the swelling behavior of this material, bacterial solutions increase the unconfined strength. Additionally, Liu et al. [24] described the effect of bio-cementation in desiccation cracks, indicating that the flocculation of clay particles forms larger aggregates. They concluded that this can be attributed to Ca^{2+} activity, which replaces monovalent metallic ions surrounding the clay particles, as well as to bacterial activity that promotes the reaction of calcium ions with minerals. The effect of stabilizing clayey soils is also shown by Chittoori et al. [25,26], who evaluate the stimulation of native bacteria in soils, applying nutrients and calcium chloride by injection. They measured the effect on swelling potential, finding that free swelling was reduced by about 50% at the laboratory level and 90% at the field scale. Morales et al. [27] showed that the precipitation of calcium carbonate increases the plastic index and the specific surface. Additionally, the precipitation generates a more aggregate structure in the soil. They found a slight increase in the friction angle and lower compressibility in soils with phyllite clays.

MICP has shown a promissory effect on soil behavior. The injection of solutions brings cohesion, increases stiffness and strength, and reduces the swelling potential. However, the impact of applying solutions within compaction water has not been widely studied. This application method can be more appropriate for landfills and fine soils because its low permeability does not allow a uniform distribution of cementing solutions by injection. Morales et al. [28] studied the effect of low proportions of carbonate (between 1 and 2%), including bacteria in compaction water of silty/clayey sands naturally containing calcium and urea. Measurements with Bender Element show that bacterial activity increases the stiffness. The efficiency of this approach depends on the availability of nutrients and Ca^{2+} in the soil. Alternatively, Chittoori et al. [26] stimulated existing bacteria in soils with expansive clays, applying nutrients within compaction water. Then, they left the sample to dry and mixed it with calcium sources in the compaction water, finding that unconfined compressive strength increased.

In this study, we investigated the influence of MICP applied within compaction water in two soils: (i) residual silty sand, and (ii) soil with expansive clays. Residual silty sand, known as *Maicillo*, is a common material on the central coast of Chile, with competent mechanical properties in its undisturbed state. Nonetheless, when remolded and compacted for landfill and embankment construction, the soil loses its cohesion and stiffness. Thus, here we studied the effect of adding MICP triggering agents to compaction water to improve the stiffness and cohesion of *Maicillo* silty sand. On the other hand, the soil with expansive clays studied were from the north of Santiago City, a zone with significant recent urban expansion of low-rise housing projects. The impact of the MICP on free swelling was studied in laboratory conditions, as well as through a field test.

2. Materials and Methods

2.1. Biotechnological Solutions

The focus of this research was the study of the geomechanical behavior of compacted soils treated with MICP. We used a biotechnological product named BAC, developed by Domolif SpA, a Chilean company. BAC is composed of bacteria that belong to the Sporosarzina species and their nutrients (see Figure 2). The product is available in two formats: (i) bacteria cultivated for 18 h in a liquid solution, then applied to the soil (henceforth referred to as CMICP), and (ii) bacteria lyophilized in the form of dry powder and reconstituted in a liquid solution in the laboratory or in the field (referred to as LMICP). Lyophilized bacteria are reconstituted, dissolving the powder in water together with industrial sodium ashes. This format simplifies the application in the field because biotechnological processes are executed separately from the construction process.

(**a**) (**b**)

Figure 2. Biotechnological product BAC: (**a**) cultivated bacteria with nutrients; (**b**) lyophilized bacteria.

The cementing product that brings the calcium ions to obtain calcium carbonate (henceforth referred to as REC) is essentially composed of calcium chloride (see Figure 3).

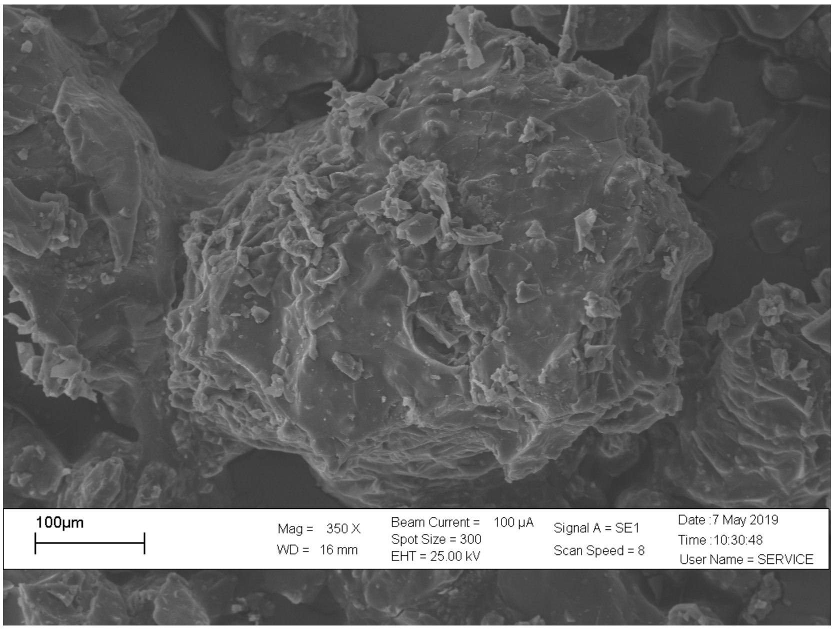
Figure 3. Calcium chloride.

To define the precipitation rates and the $CaCO_3$ content as a function of the solutions applied to the soil, we developed a set of in vitro tests that dissolved each product with the concentrations shown in Table 1 in 200 mL of water.

Table 1. Concentrations of $CaCO_3$ for in vitro tests.

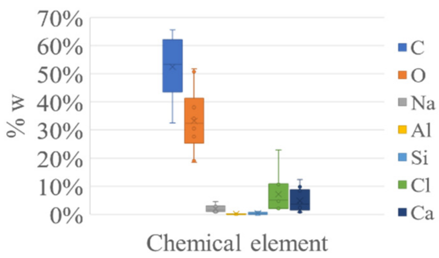
Bacteria	ID	BAC (g/L)	Ash (g/L)	REC (g/L)
Cultivated	CMICP	16	0	24
Lyophilized	LMCIP-1	16	6	24
	LMICP-2	32	12	48
	LMICP-4	64	24	96

Then, 25 mL of BAC and REC dissolved were mixed to obtain a precipitated material. The precipitate was oven-dried for 24 h at 100 °C and then weighed. One sample of LMICP-1 was triturated and disaggregated to analyze its chemical composition through an energy-dispersive X-ray spectroscopy test (EDX) and observed with scanning electron microscopy (SEM). Another fraction was triturated to analyze the mineralogical composition through X-ray diffraction analysis (XRD) with a Bruker D8 advance diffractometer, taking as a base the analysis developed with the results obtained through EDX. Figure 4a shows that the bioproduct had a dominant proportion of carbon and oxygen; however, the proportion of calcium was lower than carbonate. The XRD was analyzed with the Rietveld refinement through the software TOPAS. Since a high proportion of the precipitated calcium carbonate is amorphous, it was necessary to remove the background base line to study the peaks from the diffraction pattern. The results indicate that the crystalline fraction precipitated was calcite, and there was presence of NaCL in the form of halite and a small fraction of quartz. Due to the significant fraction of amorphous $CaCO_3$, a characterization with a Bernard calcimeter was carried out, following the procedure described in [29] for triturated LMICP-1 and in a sample of CMICP prepared following the same protocol described previously. The proportions of $CaCO_3$ precipitated in comparison to the water of the solutions preparation for LMCIP and CMICP are shown in Figure 4d.



(**a**)

Figure 4. *Cont.*



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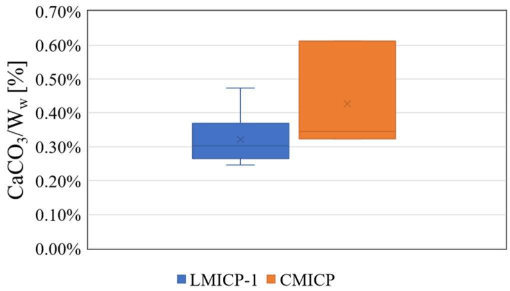
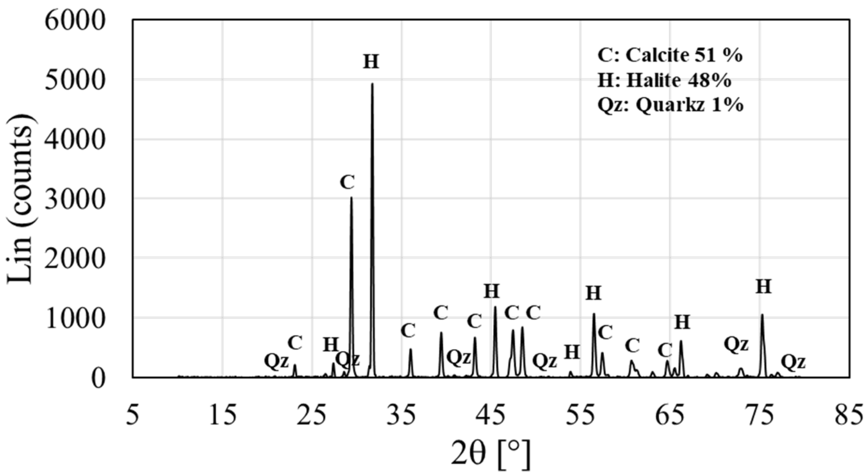


(**c**)



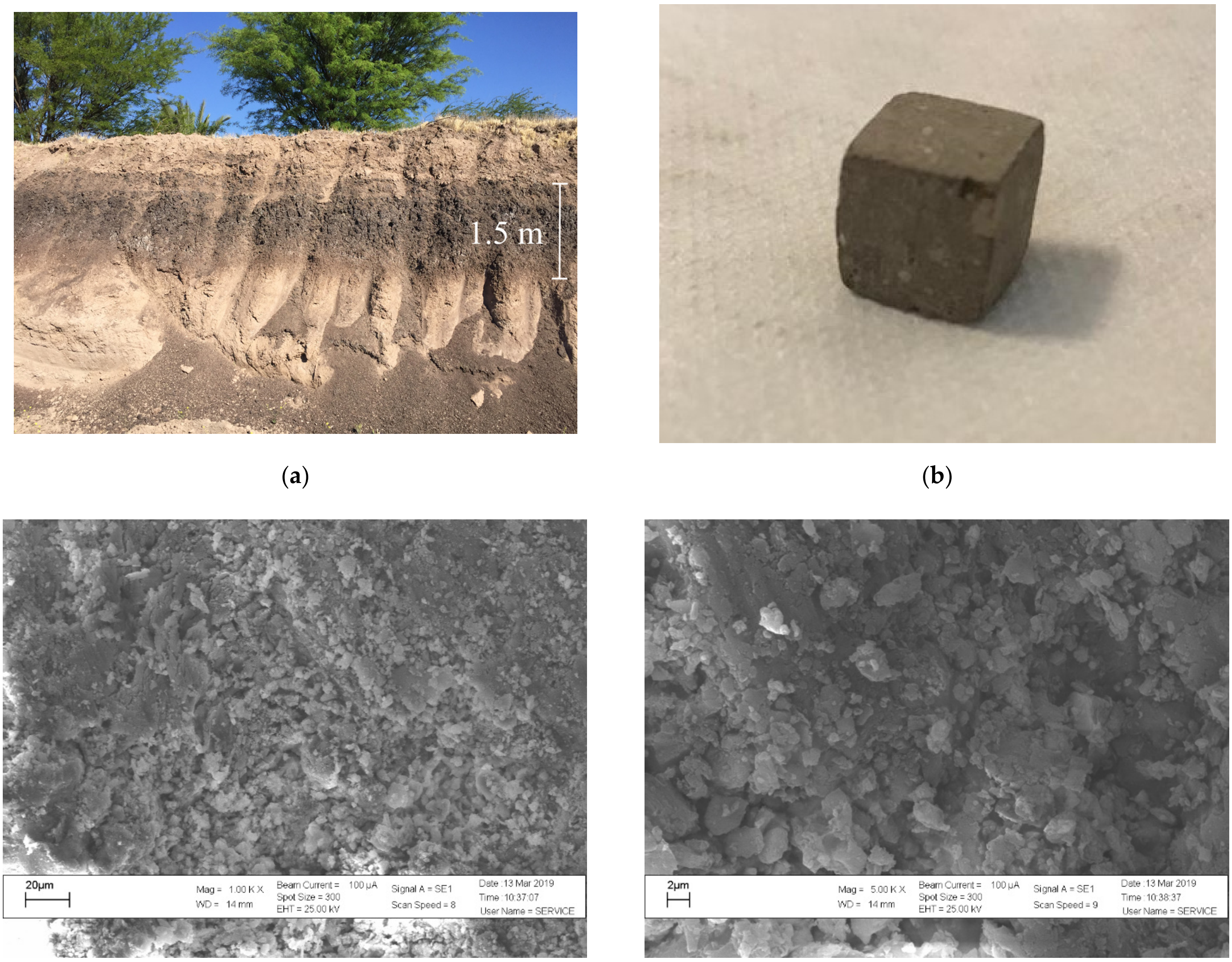
(**d**)

Figure 4. Characterization of the bio-product: (**a**) SEM image; (**b**) energy-dispersive X-ray spectroscopy results; (**c**) proportion of $CaCO_3$ in LMICP-1 and CMICP solutions; (**d**) X-ray diffraction.

2.2. Soils Description

2.2.1. Lampa Clay

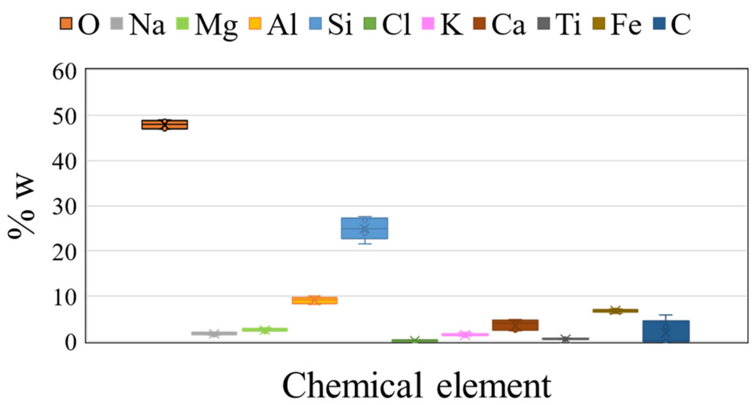
Northern Santiago is characterized by extensive deposits of soils with expansive minerals [30], causing several problems for the construction of low-rise projects due to costs of soil replacement over large surfaces. Due to the low permeability of fine soils, the application of MICP through injection or infiltration is not an easy process. However, remolding and compacting the material, including previous mixing with biological agents, could reduce the expansion and avoid soil replacement. The method should ensure a uniform agent distribution within the material to achieve a successful MICP. Soil with expansive minerals used in this project was extracted from the Lampa area (see Figure 5a) in the form of undisturbed cubes. The selection of the material was performed as a function of the Atterberg limits. To determine the composition of the soil with expansive clays and its mineralogy, we remolded a sample to form a 2 cm^3 cube (Figure 5b), which was scanned by SEM, as shown in Figure 5c,d, where the arrangement of clay particles can be observed. The chemical composition and the mineralogical analysis are shown in Figure 6. The chemical composition was defined through EDX analysis (Figure 6a), and the mineralogical composition was determined through XRD analysis (see Figure 6b), where the presence of smectite confirmed that the soil contained expansive minerals (i.e., about 58% of smectite). XRD test was carried out in a Bruker AXS model D2 Phaser diffractometer, with a molybdenum tube of 0.4 × 12 mm and a resolution of 0.01° in 1.25 s. The tests were carried out on a pulverized soil sample on a total time of 3 h and 7 min, in a range between 5° and 90°, and processed following a Rieltveld analysis with the software Profex [31].



(**a**) (**b**)

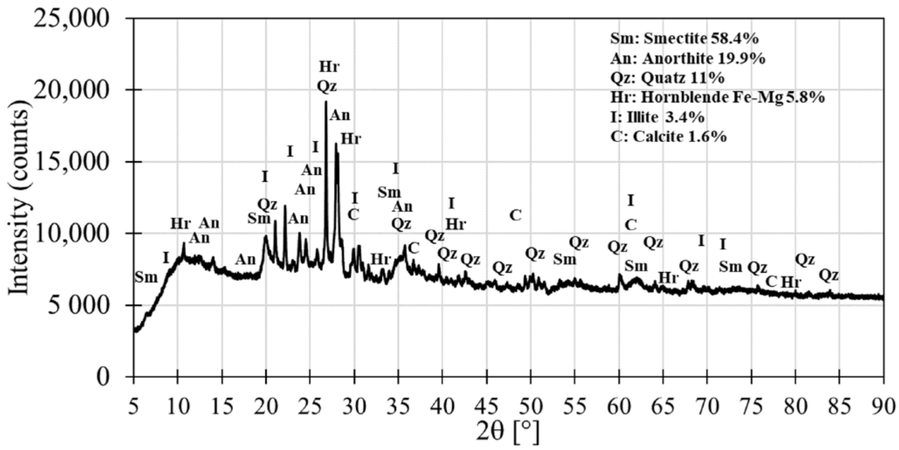
(**c**) (**d**)

Figure 5. Soil with expansive clays from Lampa: (**a**) soil profile in extraction point; (**b**) sample prepared for microscopic analysis; (**c**) SEM image 1 k X; (**d**) SEM image 5 k X.



(**a**)

Figure 6. *Cont.*



(**b**)

Figure 6. Chemical and mineralogical composition of Lampa clay: (**a**) EDX results; (**b**) XRD analysis (R_{wp} = 2.8 GoF = 2.3).

2.2.2. *Maicillo* Silty Sand

On the other hand, along the Chilean central coast there is a high presence of residual sandy soil known as *Maicillo*, formed by weathered granitic rocks (see Figure 7a), which is frequently used for landfills and embankments. Once remolded, *Maicillo* loses its cohesion and is vulnerable to surface erosion [32]. Additionally, its stiffness and strength are reduced compared with its undisturbed condition [33]. Moreover, in this case, the application of MICP-triggering agents to compaction water could provide a solution for geotechnical engineering purposes. The material used for this investigation was extracted from Quilpué city, were *Maicillo* shows a weathering profile from a fresh diorite rock with biotite, amphibole, and feldspar to a soil with biotite, albite, FeO_2, and some layering of quartz [34]. This mineralogical composition was confirmed with an XRD analysis, following the same procedure described for the sample of Lampa clay (Figure 7).

The characterization programs for both soils were (i) particle size distribution test following ASTM D6913M-17 [35], (ii) Atterberg limits according to ASTM D4318 [36], and (iii) determination of compaction properties with modified Proctor test following ASTM D1557−12 [37]. In the case of soil from Lampa, the free swelling potential was measured following ASTM D4546−14 [38] in samples compacted at 80% and 100% of maximum Proctor dry density at optimum water content. The stiffness of *Maicillo* was measured using Bender Element once the soil was compacted at 90% of maximum Proctor dry density. The mechanical behavior was described on the basis of a set of isotopically consolidated and drained triaxial tests at confining pressures of 50, 100, and 200 kPa, following ASTM D7181−20 [39], in samples at 80% and 90% of maximum Proctor density γ_{dmax}. The results of the geotechnical characterization are shown in Table 2.

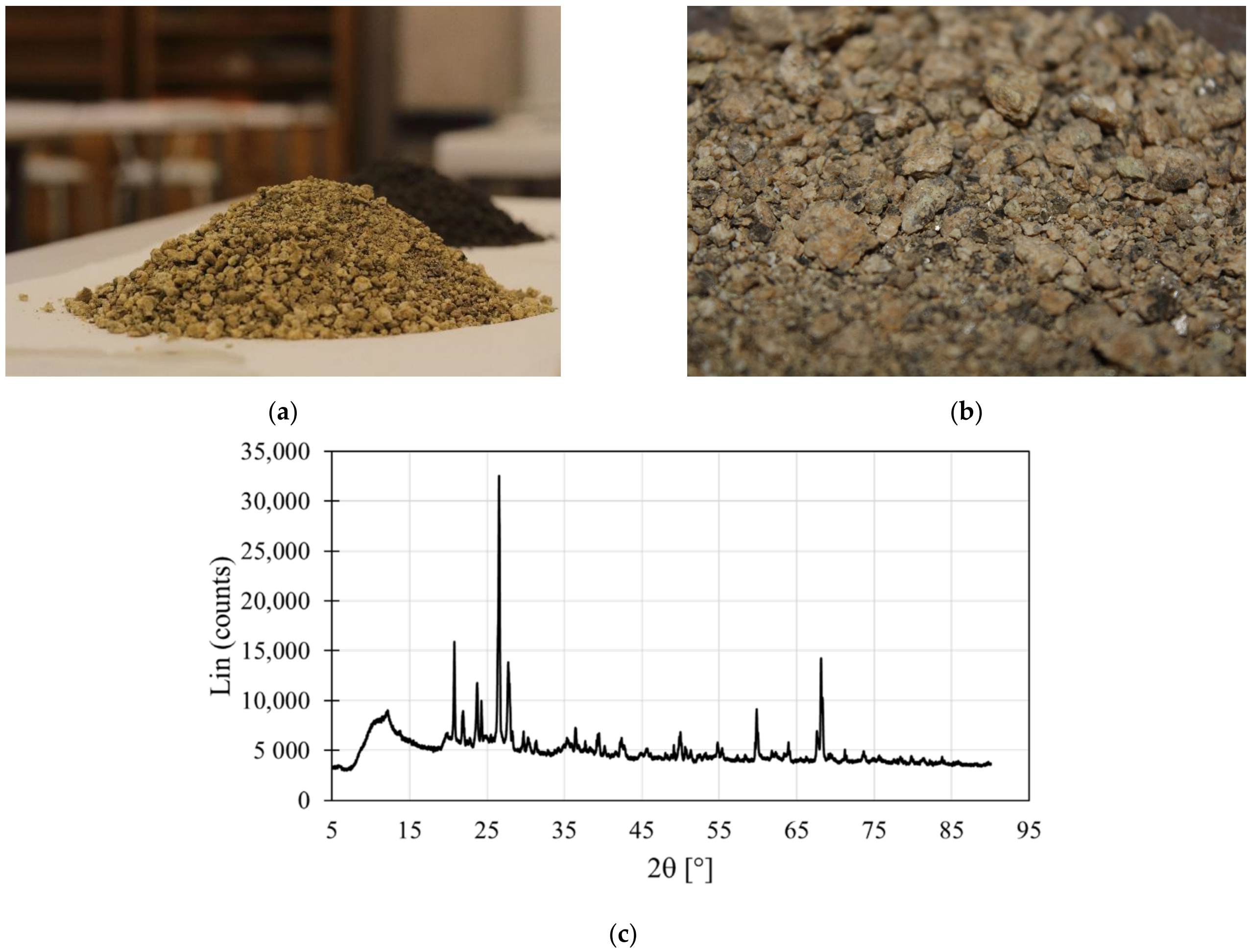


Figure 7. Maicillo from Quilpué city: (**a**) laboratory scale; (**b**) detailed view of Maicillo; (**c**) XRD analysis on *Maicillo* (R_{wp}= 6.4 GoF = 4.4).

Table 2. Characterization of soils.

Soil	*Maicillo*	Lampa Clay
Gravel	0.5%	0%
Sand	87.5%	13%
Fine content	12%	87%
Liquid limit, LL	36%	78%
Plastic index, IP	11%	49%
USCS	SP-SM	CH
Gs	2.75	2.48
Maximum Proctor density	19.52 kN/m^3	16.57 kN/m^3
Optimum water content	10%	20%

2.3. Application of MICP and Experimental Program

The treatment was applied at the optimum water content divided into two halves. We added REC dissolved in the first half of water to the soil and then mixed it until the soil was uniformly wet; next, BAC was dissolved in the second half of the water and then added to the soil mixture.

The samples treated with CMICP were compacted at 80% to 100% of the maximum Proctor density and then stored in a wet chamber under 28 °C and a relative moisture close to 100%. For both soils, samples were tested at 7 and 28 days after the treatment in order to evaluate the development of the microbial activity as a function of time. On the other hand, samples treated with LMICP were stored in an incubator at 28 °C for 15 days. The evolution of stiffness in *Maicillo* was measured at 7 and 28 days under a constant moisture content ω = 10%.

Shear strength for *Maicillo* treated with LMICP was measured in two ways: using isotopically consolidated and drained triaxial tests (saturated samples) and performing a triaxial compression test at constant water content ω = 10% (i.e., under total stress control).

Table 3 summarizes the experimental program developed. Each test condition was identified with the kind of treatment applied (CMICP: cultivated bacteria, or LMICP: lyophilized bacteria). For lyophilized bacteria, we named each treatment as LMICP-X, where X represents how many times is the solution concentration with respect to the case LMICP-1 shown in Table 1, which is the concentration of CMICP. The soils without treatment were identified as NT.

Table 3. Laboratory tests for treated samples.

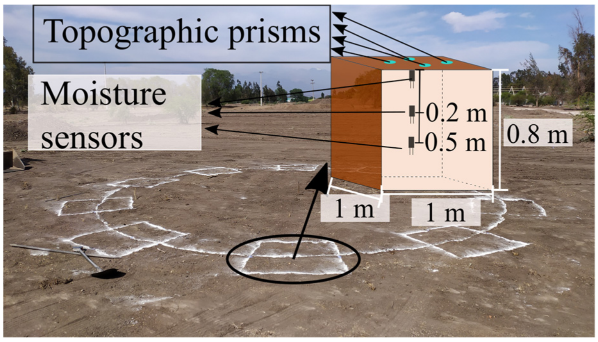
<table>
<tr><th>Soil</th><th>Treatment ID</th><th>Curing Time</th><th>% Maximum Proctor Dry Density</th><th>Test</th></tr>
<tr><td rowspan="2">Lampa clay</td><td>CMICP</td><td>7 and 28 days</td><td>100%</td><td rowspan="2">Free swelling/swelling pressure</td></tr>
<tr><td>LMICP-8
LMICP-20</td><td>15 days</td><td>80%</td></tr>
<tr><td rowspan="3">Maicillo</td><td>CMICP</td><td>7 and 28 days</td><td>80%</td><td>CID triaxial test</td></tr>
<tr><td>LMICP-20
LMICP-4</td><td>0 to 7 days
0 to 28 days</td><td rowspan="2">90%</td><td>Bender Element test</td></tr>
<tr><td>LMICP-10
LMICP-10</td><td>15 days</td><td>CID triaxial test
Triaxial compression test</td></tr>
</table>

2.4. Field Test on Lampa Clay

The effectiveness of the treatment on soil with expansive clays was also evaluated in a field test in northern Santiago. In these tests, the soil was treated with LMICP within compaction water and dissolved in the concentrations shown in Table 4. An approximate area of 20 m^2 was divided into 10 parcels of 1 $\times$ 1 m^2. Each parcel was instrumented with 3 moisture sensors at 0.0, 0.2, and 0.5 m depth to control the evolution of the water content in the soil, along with a system of automated sprinklers. In addition, four topographic prisms were installed on each parcel, and a laser topographic station with a precision of $\pm$0.1 s and 0.01 mm was used to measure the vertical displacements. Finally, two sensors of temperature and air humidity monitored the ambient conditions. The experimental setup is shown in Figure 8.

Table 4. Proportions of biotechnological products for field test.

Soil Parcel	ID	Concentrations BAC (g/L)	Ash (g/L)	REC (g/L)
P1–P2	UT	0	0	0
P3–P4	LMICP-50	800	300	1200
P5–P6	LMICP-75	1200	450	1800
P7–P8	LMICP-100	1600	600	2400
P9	REC-100	0	0	2400
P10	BAC-100	1600	600	0



(**a**)

(**b**)

Figure 8. Field test setup: (**a**) array of soil parcels; (**b**) instrumentation of soil array.

The procedure for the construction consisted of digging each parcel; then, excavated soil was left to dry at environmental conditions (between 10 and 40 °C). Next, the soil was mixed with the biological agent at the specified concentrations and then compacted at 80% of maximum Proctor density. After compaction, the soil was left for 15 days to wait for the treatment curing and to obtain a relatively dry state that maximizes swelling. At the end of the field test, soil samples were extracted to study the efficiency of the treatment in the laboratory.

3. Results and Discussions

3.1. *Maicillo*

3.1.1. *Maicillo* Treated with CMICP Compacted at 80% of Proctor Density

The first stage of treatment on *Maicillo* was developed with CMICP and soil compacted at 80% of γ_{dmax}. The samples were tested at 7 and 28 days after the application of the treatment. Figure 9 shows the deviatoric stress–axial strain (q-ε_1) path, where under low confinement (50 kPa), the secant soil stiffness at 2% of strain (E_{sec}) increased about 65% at 7 and 28 days. However, as confinement increased, the effect of bio-cementation was less significant at 7 days (negligible at 100 kPa), and it vanished at 200 kPa. Nevertheless, at 28 days, the stiffness increased by at least 13% in all cases, as shown in Table 5.

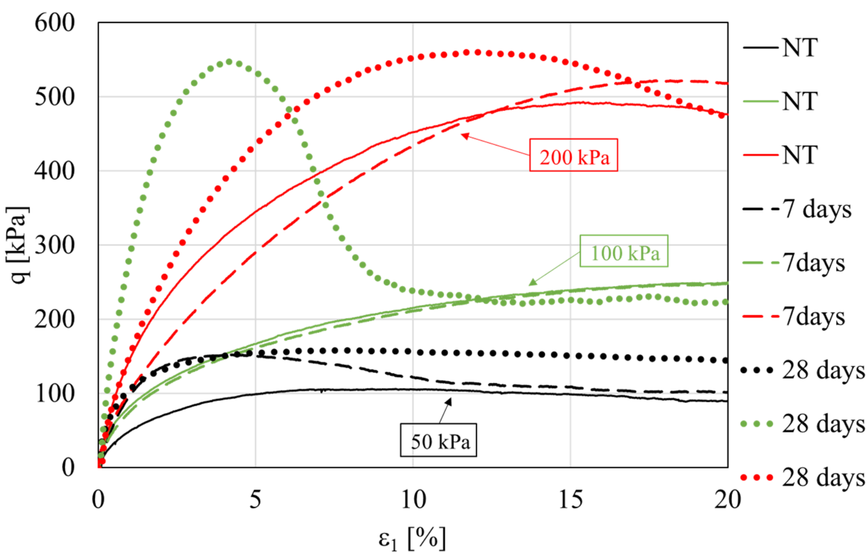


Figure 9. Triaxial for *Maicillo* treated with CMICP.

Table 5. Stiffness of *Maicillo*.

Confinement (kPa)	Case	E_{sec} (MPa)
50	NT 7 28	4 6.7 6.5
100	NT 7 28	5.6 5.4 22.2
200	NT 7 28	11 7.9 12.5

According to Table 5, the results obtained at 28 days and at 100 kPa were the most promising, as they showed a significant increase in stiffness and a very clear strength peak. Nevertheless, the stiffness evolution was erratic. Therefore, even when improvements were observed, the results tended to be within the material's dispersion, which could be related to sample heterogeneity of the residual soil.

Figure 10 shows a progressive increase of the peak strength in terms of slope of the Mohr–Coulomb envelope (MCe). The peak shear strength after seven days of curing time increased by 43%, and after 28 days, by 49% on average. Curing time is a fundamental factor in the effect of bio-cementation. Although an increase in strength and stiffness of soil was evidenced after seven days of curing, the best performance occurred after 28 days but with a significant scatter, as shown for the peak stress state at 100 kPa, compared with 50 kPa and 200 kPa of confinement. Even though a peak stress was reached in the sample confined at 100 kPa after 28 days of treatment, it is not possible to talk about a modification on the MCe because, even though the results at 50 and 200 kPa increased the strength, they were not compatible with the increase under 100 kPa.

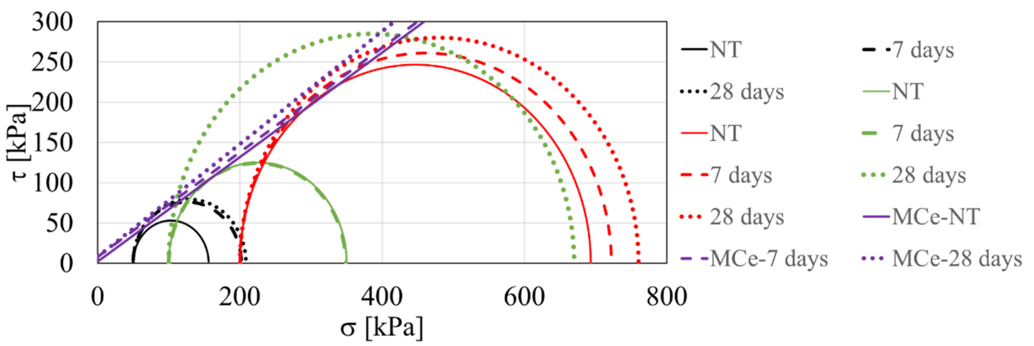


Figure 10. Mohr envelopes of peak strength.

3.1.2. Effect of LMICP on Small-Strain Stiffness

To quantify the effect of the increase in stiffness at low strain on the soils treated with lyophilized bacteria, we measured the compressive and shear wave velocities (v_p, v_s) through the Bender Element test during curing time under constant water content and confinement pressure of 50 kPa. The first sample was cured for seven days, a period in which both shear and compression waves increased (continuous lines in Figure 11). Then, the sample was saturated, and the stiffness of the soil dropped drastically. Subsequently, part of the stiffness was recovered, but a smaller portion in relation to the behavior before saturation.

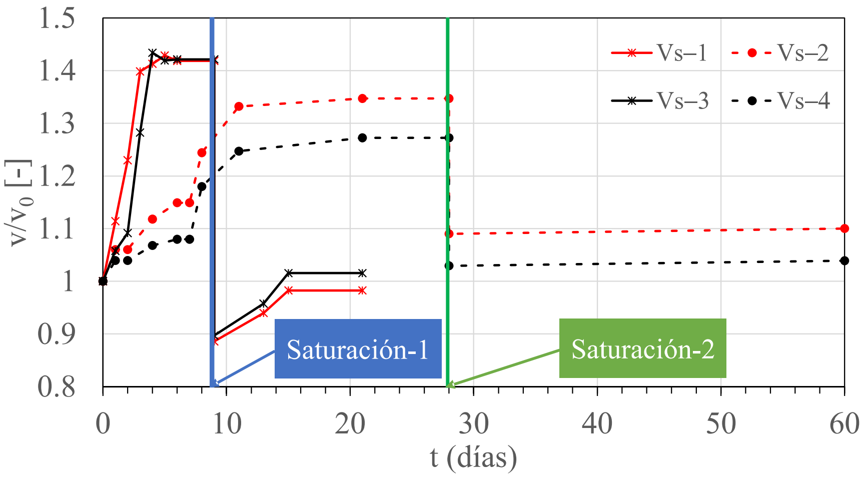


Figure 11. Wave velocity evolution in *Maicillo* treated with MICP.

Additionally, knowing that curing time is a determinant factor for the effect of bio-cementation, we decided to repeat the test with a curing time of 28 days (dashed lines in Figure 11). Although the curing time increased, the loss of the stiffness persisted once the soil was saturated. This result clearly shows a significant limitation of the bio-cement generated by LMICP.

3.1.3. Maicillo Treated with LMICP Compacted at 90% of Proctor Density

Drained triaxial tests on saturated samples of Maicillo treated by LMICP were conducted at 15 days of curing time because the Bender Element test showed that the soil reached stable stiffness (see dashed lines in Figure 11). The treatment applied was scaled to explore the effect of different concentrations of lyophilized bacteria and cementing solutions. Figure 12 shows the paths of Maicillo treated with LMICP-4 and LMICP-10. The results confirmed that saturation practically destroys any favorable effect of LMICP, even by increasing its concentration. Unlike the result for CMICP, no trend in terms of increase in stiffness or shear strength was observed. This behavior might suggest that the main structure of calcium carbonate precipitated in the soil treated with LMICP had a low proportion of calcite and high proportions of amorphous calcium carbonate.

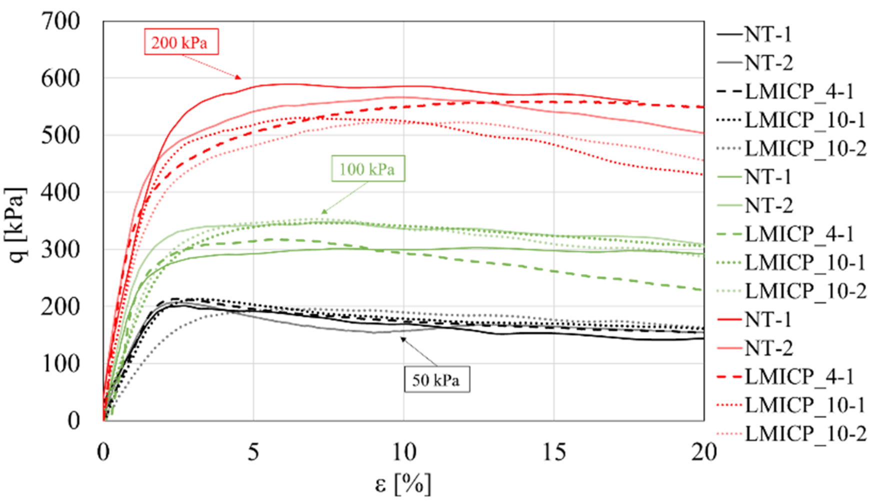


Figure 12. Drained triaxial results on saturated samples of *Maicillo* treated with LMICP.

To quantify the effect of LMICP on q-ε_1 path on unsaturated samples, we carried out triaxial tests under total confining stresses of 50 kPa at constant moisture content. Figure 13 summarizes the stress–strain path from these tests. In this case, *Maicillo* systematically increased its peak strength by about 17%. The stiffness increased with erratic behavior, resulting in a variation of E_{sec} (stiffness at 2% strain) in a range of 18% to 31%.

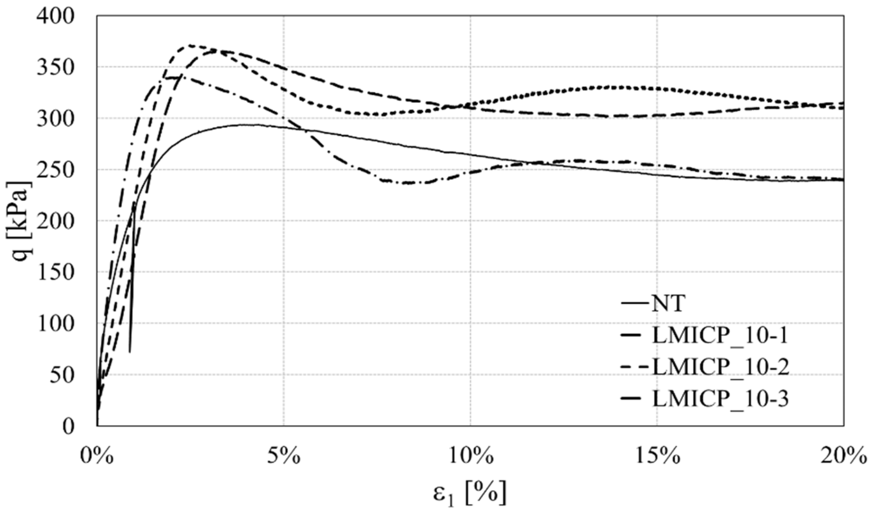


Figure 13. Triaxial compression at 50 kPa of total stress in *Maicillo* treated with LMICP.

3.1.4. Discussion of Results in *Maicillo* Sandy Silt

Results suggest that the effectiveness of treatment with LMICP is lower than CMICP. Some tests carried out on *Maicillo* treated with LMCIP, using the same concentrations as applied to CMICP, did not show any effect in the soil behavior under saturated conditions, and despite an increase in the concentration of biotechnological solution, the change was clear only under unsaturated conditions with concentrations 10 times greater than CMICP. One of the possible reasons is that in the lyophilization process the bacteria (BAC) is stored with maltodextrin (widely used to protect bacteria and proteins during spray drying), which can encapsulate the calcium carbonate, reducing the effectiveness in the generation of bonds between particles.

Concerning the amount of precipitated $CaCO_3$, our results suggest that the quantity of $CaCO_3$ precipitated is not enough to significantly improve the mechanical behavior of *Maicillo*. In fact, Cui et al. [40] concluded that low proportions (less than 1% of calcite content) are not significant for improving soil properties Figure 14 illustrates a quantification of precipitated $CaCO_3$, obtained from the mass of soil and water, and the proportions measured through a Bernard calcimeter. It can be concluded that the precipitation obtained in this study was below 1%. Low proportions of precipitated calcium carbonate cannot generate stable structures; they need to be supersaturated in order to evolve into better structures [41].

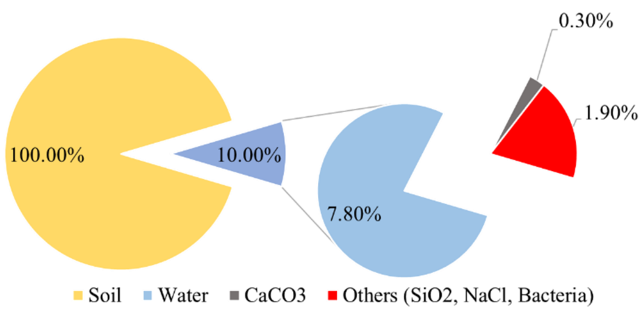


Figure 14. Proportion of $CaCO_3$ in soils.

Morales et al. [28] evaluated the generation of $CaCO_3$, using calcium and nutrients naturally present in the soil and adding the bacteria into the compaction water. They achieved an increase in the $CaCO_3$ content from 1.1% to 1.8%. As a result, they reported a moderate increase in the shear strength and small-strain stiffness under unsaturated conditions. On the other hand, Chittoori et al. [26] mixed the bacterial solution and left bacteria to develop the hydrolysis during different periods of time; they left the soil drying and then compacted the soil, dissolving the cementitious solution into the optimum water content and measuring the unconfined compressive strength. They found that the increase in shear strength was not relevant, which is similar to the results that we show in Figure 10, concluding that there is a need to evaluate the bacteria in terms of its ability to precipitate a stable structure of $CaCO_3$. Moreover, a pre-treatment should be needed with more cycles of mixing and drying with treatment solutions to reach higher proportions of calcium carbonate within the material.

3.2. Lampa Clay

3.2.1. Laboratory

Samples of Lampa clay were compacted at 100% of maximum Proctor density and stored in the incubator to be tested after 28 days of curing. Table 6 shows the results of free swelling strain (ε_v) tests on samples treated with CMICP. As shown in Table 6, the expansion of soil was reduced almost to zero, which, as described by Liu et al. [24], could be a consequence of the flocculation of clay sheets due to the effect of calcium chloride and the biocementation of calcium carbonate bonds the minerals within the soil's fabric.

Table 6. Free swelling tests on soils with expansive minerals compacted at 100% of maximum Proctor density.

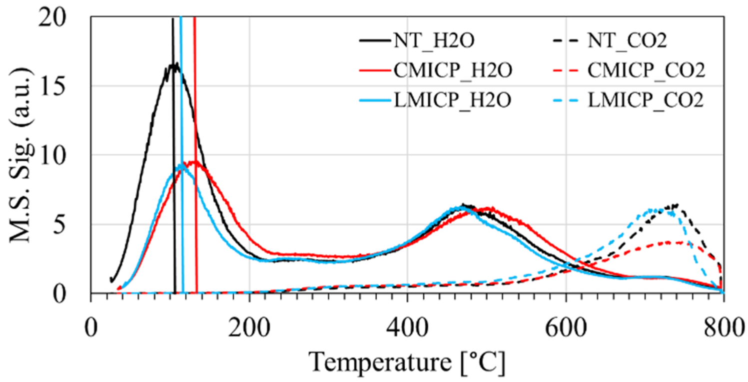
Condition	ε_v (%)
NT_1	5%
NT_2	4.7%
CMICP_1	0%
CMICP_2	0%

The tests were repeated by treating the soil with LMICP at 80% of maximum Proctor density. The density was reduced because these values were closer to the density achieved during construction in the field. The results on the untreated material showed more swelling strain compared to previous tests shown in Table 6, probably due to soil heterogeneity. Table 7 shows that LMICP treatment on soil from Lampa at the laboratory was favorable, showing in some cases a total reduction of the swelling potential. Additionally, we prepared a test with calcium chloride (REC) to assess the effect of that product on swelling potential, showing that this solution stabilizes the particles by itself. The effect of calcium chloride flocculated the clay particles, which means that the mineralogy of the soil became stabilized due to the replacement of monovalent metallic ions [24].

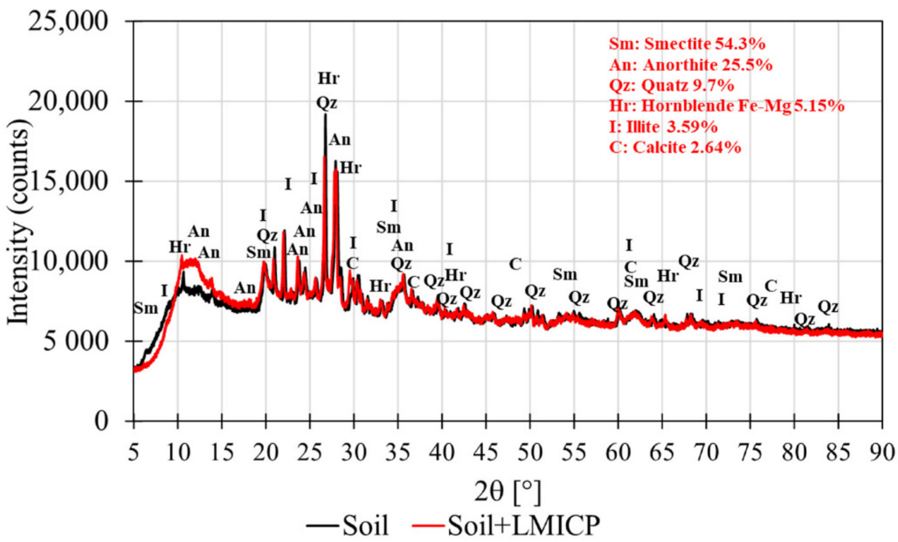
Table 7. Free swelling test on Lampa clay at 80% of maximum Proctor density.

Condition	ε_v (%)
NT_1	9.0%
NT_2	9.7%
LMICP-8_1	5.8%
LMICP-8_2	3.5%
LMICP-20_1	2.3%
LMICP-20_2	0%
REC-20_1	0.5%
REC-20_2	0%

Internal changes in the material were quantified with additional tests Figure 15 shows XRD patterns and temperature-programmed desorption (TPD), where the vertical axis indicates the atomic mass of particles evaporated (water and carbon dioxide). It can be seen that clays treated with CMICP changed the temperature of water evaporation within the soil, from 100 to 120 °C. Samples treated with LMICP changed that temperature to 110 °C. Results with TPD indicate that free water in the soil presented a resistance to evaporation in treated soils, which could have been due to the organic material generated with the CMICP and LMICP processes.



(**a**)



(**b**)

Figure 15. Analysis of treated and untreated Lampa clay: (**a**) temperature-programmed desorption, evaporation of water and CO_2 in atomic mass unit as a function of temperature; (**b**) XRD patterns (R_{wp} = 2.9 GoF = 2.4).

Furthermore, no major impact of the treatment was observed at evaporation temperatures close to 500 °C. However, more studies are needed to clarify this point. XRD (Figure 15b) observations did not provide any information about a change in the amount of calcium carbonate. The distribution of the natural calcium carbonate in the soil had variability, which made it difficult to detect additional low quantities of carbonate precipitated due to treatment.

3.2.2. Field Test

Given the favorable results at the laboratory scale, we proceeded to scale them up to field conditions. After the compaction and curing time of materials, we installed an irrigation system which was developed to keep moisture constant, according to the sensor reading. Then, the changes in the surface level were measured with the laser topographic station Figure 16 shows the evolution of vertical elevation of soil once the irrigation started; parcels P1 to P9 had an increase in the surface level with a similar magnitude. However, the surface increase registered in parcel 10 had a lower magnitude contrasted with control parcels (P1–P2).

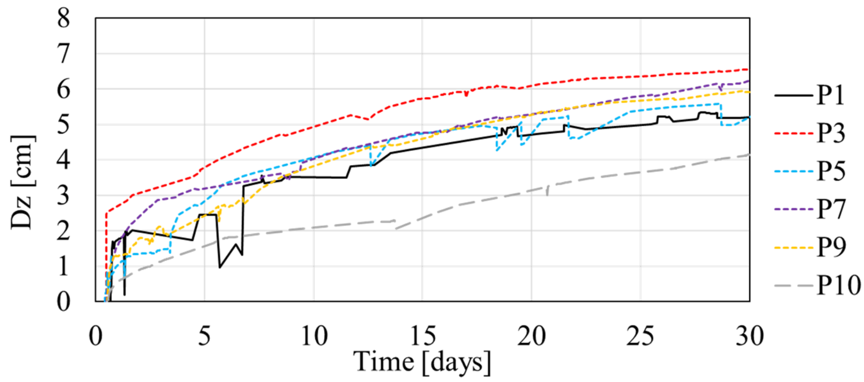


Figure 16. Change in the surface level of the soil during field test.

Unfortunately, in the field, the MICP treatments did not have the success observed in the laboratory. There are several possible explanations. First, high proportions of LMICP should increase the presence of maltodextrin encapsulating the particles of calcium carbonate. Second, during the mixing process of soils with treatment, the material became very hard to manipulate, and once REC was applied, the addition of BAC was not uniform. Third, continuous irrigation cycles could have destabilized the structure of precipitated calcium carbonate.

On the other hand, Figure 17 shows the evolution of electric resistivity in the soil parcels during the execution of the field test. The behavior of the electric resistivity showed that on treated soils (P4, P6, and P8), it was reduced in comparison with non-treated soils and also with materials treated only with BAC solution (P1 and P10). Nevertheless, the parcel treated only with REC did not show the same behavior, which indicates that the presence of calcium chloride is the main factor that generates that electrical resistivity change in the material.

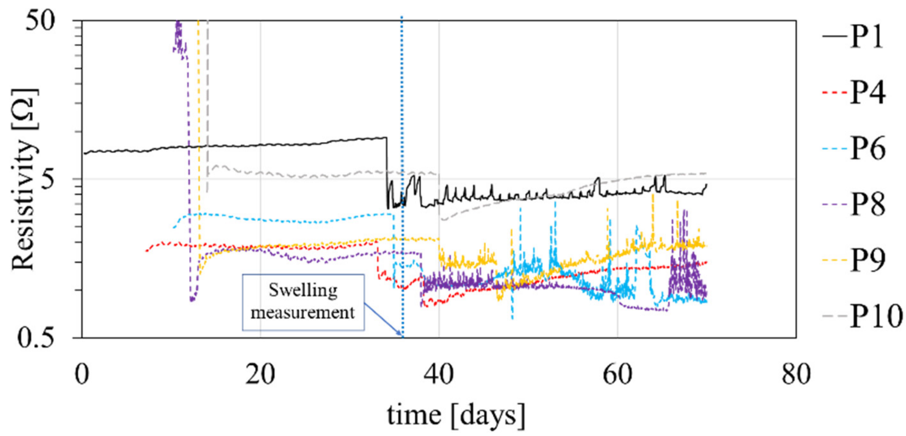


Figure 17. Electric resistivity of clay parcels.

After the measurements were finished, some samples were extracted on each parcel, and the Atterberg limits were evaluated. Table 8 shows that there were no significant changes in the samples tested. However, in parcel 10, which was treated only with BAC, there was a slight increase in the liquid limit (LL). Qualitatively the material from parcel 10 was softer than treated material from other parcels and was the only one that showed some reduction in the expansion compared with untreated parcels (P1 and P2). We believe that the increase in bacteria concentration in parcel 10, as well as the natural presence of calcium carbonate (Figure 15b), could have partially stabilized the structure of the natural soil, reducing the swelling.

Table 8. Atterberg limits in field test.

Parcel	LL	IP
1	56	26
4	56	27
6	54	27
8	55	31
9	55	27
10	60	35

3.2.3. Discussion of Results in Soil with Expansive Clay

To compare the treatment methods used in this study, we measured the amount of calcium carbonate in untreated and treated (both CMICP and LMICP) samples of Lampa clay using two techniques: XRD and Bernard calcimeter. XRD shows the proportion of calcite carbonate within all the crystalline structures presented in the material. The carbonate precipitated by bacteria tends to be amorphous and not able to be detected by this method. Therefore, the measurements of carbonate were complemented with a Bernard calcimeter, which measures the quantity of $CaCO_3$ within the total mass of soil. Both techniques are complementary.

The measurements developed through XRD showed that presence of crystalline $CaCO_3$ was 35% on average, while a mean value of 32% was obtained for CMICP, and 36% for LMICP. This result illustrates the natural variability of calcium carbonate in the material because induced calcium carbonates tend to be amorphous and are not feasible to detect by XRD. On the other hand, the Bernard calcimeter showed a variation in the untreated soil between 0.9 and 1.2% of total mass, and in treated samples, the proportions were within the same range (0.9–1.2%). This test quantifies the amount of calcium carbonate from the whole soil mass and shows non-crystalline and crystalline structures. However, it was not possible to obtain a quantitative estimate of the amount of precipitated $CaCO_3$ using this technique either, probably because the base fraction of carbonate in the material was already high and precipitation presents a marginal fraction within this total.

On the other hand, measurements with a Bernard calcimeter were performed in the samples extracted from the field test. The measurement of carbonate indicated a weight fraction of about 1% in all the field samples for both treated and non-treated materials. The lack of a significant difference in the presence of this mineral within the soil could explain the non-favorable behavior obtained in the field test.

In principle, the presence of calcium ions (2+) provided by REC could modify the behavior of the soil. However, although no effect was observed in the field test, we believe that one possible explanation is because a poor distribution of REC was obtained. REC does not affect the pH of the soil, while BAC generates an increase of pH. Changes in the pH originated by the bio-chemical processes could stabilize the natural fraction of calcium carbonate present in the soil. Since in the field the distribution of REC was not homogeneous, we believe that the humidity of this product sealed the material, preventing the action of BAC. As the laboratory mixture of REC was more homogeneous and, in the case of parcel 10 REC, was not used, we believe that this effect could also contribute to explain the difference in the results achieved.

An improvement of the soil behavior was obtained in treated soils in the laboratory. However, a more efficient treatment distribution method is needed in the field to achieve more significant proportions of calcium carbonate and thus effectively reduce soil expansion.

4. Conclusions

This paper presents a comprehensive study to evaluate the effect of MICP applied to compaction water in soils containing expansive clays (Lampa clay) and sandy silt (*Maicillo*). This method allows for a better distribution of the biological treatment, and its application is relatively simple when associated with a compaction process.

The results indicate that *Maicillo* treated with CMICP increases both stiffness and shear strength compared against results obtained with LMICP, possibly due to the presence of maltodextrin in lyophilized bacteria and because the quantity of calcium carbonate precipitated (less than 1%) is not enough to form a network of cemented contacts, affecting the macroscopic properties of the material. Moreover, scaling the proportions does not improve the effectiveness of the treatment, as it also increases the quantities of this polymer.

The treatment with cultivated bacteria (CMICP) on *Maicillo* had, in general, a better performance. However, after seven days, the effects were negligible compared to 28 days of curing time, where an increase in stiffness and strength was more evident than in seven days. Saturation after application of LMICP almost destroyed the effect of the bio-cementation, suggesting that a stable form of calcium carbonate was not achieved. Therefore, the application of LMICP requires a complementary treatment to obtain stable effects. For example, treatment solutions could be applied in several cycles of moisture and drying before compacting to obtain concentrations higher than 1% of calcium carbonate precipitated.

The presence of calcium carbonate in soil with expansive clay shows an effect in stabilizing swelling potential at the laboratory scale, reducing the swelling potential to zero. However, in the field test, the results did not show the same effect due to the poor distribution of biological agents and saturation of soil by cycles of irrigation and subsequent destabilization of precipitated carbonate. This could be improved, in principle, by applying cultivated bacteria and by increasing the number of treatment application cycles in future works.

Comparing results obtained in *Maicillo* against Lampa clay, we obtained more promising results in clay under laboratory conditions because of the highest proportion of calcium carbonate naturally available in the soil. However, a more detailed mineralogical analysis is needed in future works to establish more robust trends.

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